



# Environment and Natural Resources Trust Fund

## 2023 Request for Proposal

### General Information

**Proposal ID:** 2023-153

**Proposal Title:** Northward Expansion of Ecologically-Damaging Amphibians and Reptiles

### Project Manager Information

**Name:** Kenneth Kozak

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

**Office Telephone:** (612) 624-3982

**Email:** kozak016@umn.edu

### Project Basic Information

**Project Summary:** American bullfrogs and Red-eared sliders are non-native predators and competitors in Minnesota's native fish communities. This research will assess the distribution and potential for expansion of these species in Minnesota.

**Funds Requested:** \$163,000

**Proposed Project Completion:** July 31, 2025

**LCCMR Funding Category:** Small Projects (H)

**Secondary Category:** Aquatic and Terrestrial Invasive Species (D)

### Project Location

**What is the best scale for describing where your work will take place?**

Statewide

**What is the best scale to describe the area impacted by your work?**

Statewide

**When will the work impact occur?**

During the Project and In the Future

## Narrative

### **Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.**

Minnesota has experienced dramatically warmer temperatures in the past decades. Many species reach a northern limit to their distribution in Minnesota. To the extent that this pattern is influenced by the adaptive capacity of populations at the range edge (e.g. limited tolerance to cold), non-native species with limited ranges in Minnesota (or range limits to the south of the state), are predicted to expand their ranges northward as the climate warms.

American bullfrogs and Red-eared slider turtles are non-native species that have recently become established in Minnesota, and are two of the most pervasive and detrimental invasive species when they are introduced into waterways in which they are not native. Both species outcompete and feed on native fish species, restructuring the food webs and threatening native species with extinction. Given that fish populations contribute greatly to local community revenue and the health of the ecosystems, understanding the current and predicted future geographic distributions of these non-natives, and their impact on fish communities is critical. Although American bullfrogs and Red-eared sliders have limited ranges in Minnesota, they are expected to expand northward through the state as temperatures continue to increase with climate change.

### **What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.**

The proposed research will use environmental DNA (eDNA) and thermal-tolerance data to assess the current distributions of American bullfrogs and Red-eared sliders in Minnesota and their potential for expansion across the state.

These species are likely expanding their ranges into locations in Minnesota where they have yet to be detected. eDNA is new a tool that can document species that are in low abundance and/or have limited activity windows (such as amphibians and reptiles) by detecting traces of species' DNA left in its habitat. By collecting water samples in drainage systems immediately to the north of the currently known distributions of the focal species, we will be able to determine whether these two species are expanding their ranges beyond their currently-known range limits.

We will measure the thermal tolerances of these species at the northern range limit and in more in southern localities. Broader thermal tolerance and/or increased tolerance to cold at the range edge would be evidence of adaptive evolution to climate and more rapid northward range expansion than expected based on species simply tracking favorable climate. Thermal-tolerance data will be used to generate species-specific models of the species' current and future distributions across Minnesota.

### **What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?**

The proposed research will provide predictions of impact on Minnesota's aquatic communities by two non-native species that are notorious aquatic ecosystem invaders. The results will inform the management of aquatic ecosystems and will identify the potential threats of non-native predators and competitors to Minnesota's native fish communities. The modeling of the future distributions of American bullfrogs and Red-eared sliders will predict whether continued expansion across the state's waterways is expected in response to Minnesota's changing climate, providing a foundation for developing adaptive management strategies to protect the state's aquatic ecosystems.

## Activities and Milestones

### Activity 1: Species surveys and eDNA sample collection

**Activity Budget:** \$72,000

**Activity Description:**

We will survey for species occurrence, and collect data on body and activity temperatures for the focal species. We will also collect water samples for eDNA analyses along North-South transects in drainage systems that American bullfrogs and Red-eared sliders are presently known to inhabit, as well in drainage systems immediately to the north (e.g. Red River Basin, upper reaches the Mississippi Headwaters Basin, Lake Superior Basin) to survey for recent northward expansion.

**Activity Milestones:**

Description	Completion Date
Documentation of the distributions of American bullfrogs and Red-eared sliders	July 31, 2024
Collection of eDNA water samples from survey locations	July 31, 2024
Filtration of eDNA water samples and extraction of eDNA for qPCR	July 31, 2024

### Activity 2: Thermal tolerance and role of climate in range limits and range expansion

**Activity Budget:** \$71,000

**Activity Description:**

We measure the thermal tolerances (sensitivity and duration of activity over a range of environmental temperatures in the field) of the focal species at the northern range edge and in more southern localities. If adaption to climate constrains northward dispersal, no differences in thermal tolerance are expected between the range edge and more southern localities. Broader thermal tolerance and/or increased tolerance to cold at the range edge would be evidence of adaptive evolution to climate and more rapid northward range expansion than expected based on species tracking favorable conditions as the climate warms. Thermal tolerance data will be used to parameterize species-specific models that will map suitable habitats for the focal species across the state based on the current climate and also under predicted rates of future warming.

**Activity Milestones:**

Description	Completion Date
Determine the role of climate in restricting the northward expansion of species	July 31, 2025
Predict the current and future distributions of species based on thermal tolerance	July 31, 2025

### Activity 3: eDNA data collection and analysis

**Activity Budget:** \$20,000

**Activity Description:**

To understand the distribution of the focal species in MN we will analyze eDNA from locations where the two species were observed and surveyed, and also to the north in drainage systems where they may have expanded their ranges, but yet to have been documented. The collection and analysis of eDNA samples from drainages in which these species are already documented will serve as a positive control for the efficacy of our eDNA methodology for detecting the focal species in other locations. We will generate maps of all the drainage systems in which American bullfrogs and Red-eared sliders are detected with eDNA.

**Activity Milestones:**

Description	Completion Date
qPCR of eDNA extracted from water samples	July 31, 2025
eDNA sequence assembly and species identification of amplified eDNA	July 31, 2025
Map drainages in which the focal species were detected with eDNA	July 31, 2025

## Long-Term Implementation and Funding

**Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?**

We will communicate the results to the general public at the Minnesota State Fair and the University of Minnesota's Bell Museum. Research results will be disseminated to the scientific community through journal publications and research presentations at regional and international meetings. Future development of the proposed research will focus on broadening its scope to explore the changing distributions of rare, native species of conservation concern, and also common native species, thereby providing a means to address how aquatic communities in Minnesota may shift and become restructured with climate change.

## Project Manager and Organization Qualifications

**Project Manager Name:** Kenneth Kozak

**Job Title:** Associate Professor and Curator, Fisheries, Wildlife & Conservation Biology and Bell Museum

**Provide description of the project manager's qualifications to manage the proposed project.**

Education:

B.S. Biology, 1995, Johnson State College, Johnson, VT

M.S. Zoology, 1999, Clemson University, Clemson, SC

Ph.D. Evolution, Ecology, and Population Biology, 2005, Washington University, St. Louis, MO

Research expertise:

My research program is centered on understanding on how species spread, diversify, and accumulate across the landscape over time. To address these questions, my lab in the Department of Fisheries, Wildlife & Conservation Biology and Bell Museum collects and analyzes genetic and ecological data in a spatial framework. My most recent research focuses on identifying the evolutionary and ecological factors that limit the geographic ranges of narrowing-ranging amphibian species and predicting how these vulnerable species will respond to climate change.

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

**Organization Description:**

In the College of Food, Agricultural and Natural Resources Sciences (CFANS) we look at the bigger picture. When we envision a better tomorrow, it includes disease-resistant crops, products that protect our health, lakes free from invasive species, and so much more. We use science to find answers to the world's grand challenges and solve tomorrow's problems.

Twelve academic departments and 10 research and outreach centers make up our college, along with the Minnesota Landscape Arboretum, the Bell Museum, and dozens of interdisciplinary centers.

As part of a major urban university located in the heart of the Twin Cities, we also provide immersive study opportunities across the state. Our living laboratories allow students, faculty, and staff to study throughout Minnesota's diverse ecosystems.

## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
<b>Personnel</b>								
12-month Graduate Research Assistant		Data collection and analysis			25%	1		\$101,000
Associate Professor (2-months summer salary)		Project supervision, data analysis, dissemination and publication of results			36%	0.16		\$28,000
							<b>Sub Total</b>	<b>\$129,000</b>
<b>Contracts and Services</b>								
							<b>Sub Total</b>	-
<b>Equipment, Tools, and Supplies</b>								
	Tools and Supplies	DNA extraction kits (15 kits @ \$200/kit)	Supplies required to extract eDNA from samples					\$3,000
	Tools and Supplies	Sterlitech water filters 810kits @ \$200/kit)	For filtration of eDNA from water samples					\$2,000
							<b>Sub Total</b>	<b>\$5,000</b>
<b>Capital Expenditures</b>								
							<b>Sub Total</b>	-
<b>Acquisitions and Stewardship</b>								
							<b>Sub Total</b>	-
<b>Travel In Minnesota</b>								

	Miles/ Meals/ Lodging	Mileage to field sampling sites (2000 mi @0.585/mile)	To collect eDNA samples from MN waterways					\$1,000
	Miles/ Meals/ Lodging	GSA per diem (lodging + M&IE @ MN standard rate, 30 days @ \$155/day),	Costs for meals and lodging associated with travel to field sites to collect samples.					\$5,000
							<b>Sub Total</b>	<b>\$6,000</b>
<b>Travel Outside Minnesota</b>								
							<b>Sub Total</b>	-
<b>Printing and Publication</b>								
	Publication	Page charges to publish in open-access scientific journal	Dissemination of research results to scientific community					\$3,000
							<b>Sub Total</b>	<b>\$3,000</b>
<b>Other Expenses</b>								
		Lab services: qPCR @ University of Minnesota Genomics Center (800 samples @ \$25/sample)	Analysis of eDNA from water samples					\$20,000
							<b>Sub Total</b>	<b>\$20,000</b>
							<b>Grand Total</b>	<b>\$163,000</b>

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
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Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
<b>State</b>				
			<b>State Sub Total</b>	-
<b>Non-State</b>				
			<b>Non State Sub Total</b>	-
			<b>Funds Total</b>	-

## Attachments

### Required Attachments

#### *Visual Component*

File: [9e01fcab-1d1.pdf](#)

#### *Alternate Text for Visual Component*

Known county-level distribution for American bullfrogs and Red-eared slider turtles in Minnesota...

## Administrative Use

**Does your project include restoration or acquisition of land rights?**

No

**Does your project have potential for royalties, copyrights, patents, or sale of products and assets?**

No

**Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?**

N/A

**Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?**

N/A

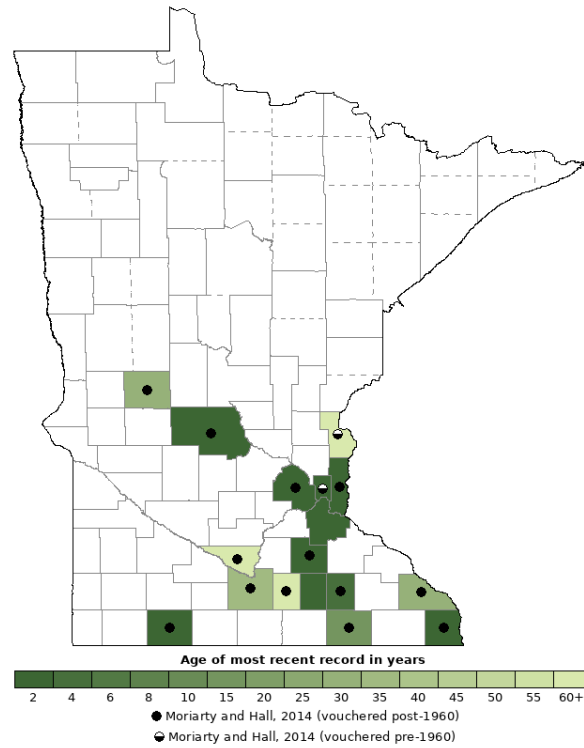
**Does your project include original, hypothesis-driven research?**

Yes

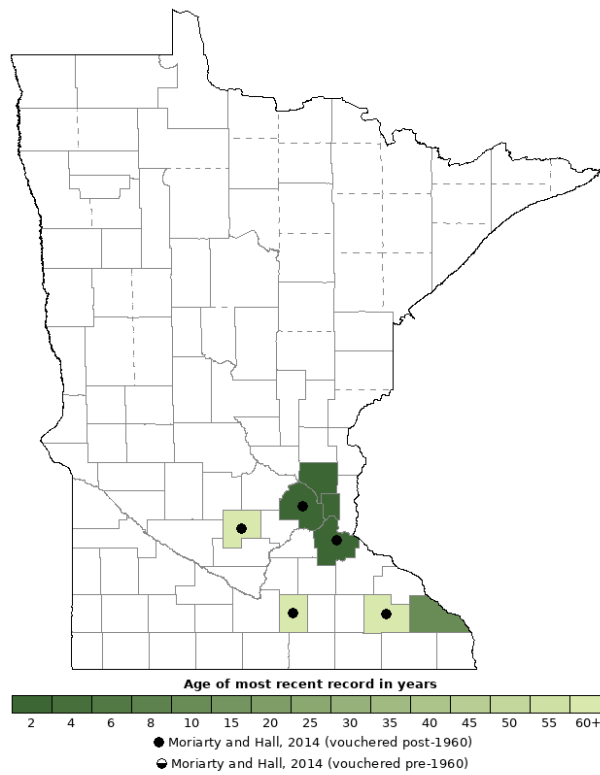
**Does the organization have a fiscal agent for this project?**

Yes, Sponsored Projects Administration

(A)



(B)



County records for (A) the American bullfrog, *Lithobates catesbeianus*, (B) the Red-eared slider turtle, *Trachemys scripta*, in Minnesota. The maps are generated from data from the Bell Museum (<https://bellatlas.umn.edu>) and HerpMapper (<https://herpmapper.org>).

